

Summary: Conference on Dibenzodioxins and Dibenzofurans, National Institute of Environmental Health Services, April 2-3, 1973

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To attempt to summarize the proceedings of a meeting such as this one in any rigorous sense is clearly an unreasonably ambitious task. It is made more challenging because of the tentative character of much of the work commented upon here.

This meeting, one of a series (and I hope a growing series) for the National Institute of Environmental Health Sciences, represents an extraordinarily useful concept. It is designed to bring together a variety of scientific bedfellows who have contemplated (and indeed investigated) a subject for its academic interest and for its very timely topical interest. These same scientists would have learned about each other eventually but, in the best tradition of science, it would have taken a long time.

By design, we are witnessing the cutting edge of scientific research for this area. This meeting has, as its avowed and very virtuous purpose, the calling out of the walls a good deal of unmaturing and not totally interpreted or confirmed research. It is important to keep this tentative and unconfirmed character in mind.

One is struck, too, by the character of the research which has been reported at this meeting. There are still many gaps in our knowledge about dibenzofurans and dioxins.

Yet, the thought that has gone into the design of the research reported here represents a striking degree of sophistication in many cases for which the participants should be very proud. As late as 1970 or 1971, there existed only the crudest hint of a ranking of biological activity of members of the family of chlorinated dioxins. (1, 2) The reports at this conference contained descriptions of dose-response information, some beginning insight into mechanisms, and the first probings toward structure-activity relationships. I am struck by the fact that the early observations (from the occupational environment) and the early approximations of rankings have been sustained and essentially confirmed by the data reviewed at this meeting.

Chemistry, Analysis, and Chemical and Physical Properties

Dr. Langer's paper on the formation of dioxins from precursors through condensation reactions represents a good example of what we should do more of. I speak here of the attempt to predict probable and improbable behavior in the environment from knowledge of physical and chemical properties. Dr. Langer's nuptial analogy is apt in more than one way. He reminded us that chemical courtship leading to marriage (in this case, condensation) was family-specific

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and adhered to some orthodox rules. Family character, and how this is perceived by the other party to the arrangement, seems to be important. It seems to me that Dr. Langer has thrown down the challenge of confirmation for dioxin formation, reported on by some as feasible in the environment. I am sure that this challenge will be taken up.

One other point was raised by Dr. Langer and was echoed independently by others was the spurious formation of condensation products within the chambers of the very instruments used to detect them (e.g., the gas chromatograph).

What is the evidence of "weathering"—the formation in nature of dioxins or furans from chlorinated materials through the addition of energy from somewhere? Crosby et al. and Hutzinger et al. suggested that condensation reactions to form dioxins or dibenzofurans might be promoted by exposure to sunlight and presented the results of a few preliminary experiments to examine this subject. What they properly reminded us of, however, was the fact that the story does not end there. The presence of detectable condensation products depends on the dynamics of both production and ensuing decomposition. Decomposition, they reminded us, occurs typically through reduction and here depends on an available hydrogen source. (They demonstrated the point in their laboratory experiments by using a hydrocarbon medium.) Tetrachlorodibenzo-*p*-dioxin was found to be more labile than the octochlorinated member of the family. It was speculated that there was sufficient organic material in most environmental situations to assure hydrogen donors. In brief, environmental persistence seems unlikely. The evidence seems to suggest that those impurities which are found are of the less toxic varieties. However, we need more samples because of the large variety of commercial products.

A. E. Pohland et al. revealed some of the potential and the limitations of two analytic methods, electron spin resonance and visible light spectroscopy. As I heard this paper, these sounded particularly useful as con-

firmatory techniques. The authors cited the need for pure standards to realize the potential of their methods.

Dr. Crummett's paper is perhaps the latest in a growing series of examples of how the power of analytic methods tends to "drive" manufacturing procedures to be more rigorous and produce greater degrees of purity. His point is well made. We are all better off as a result.

It is heartening to note the similarity in degrees of resolution reported both by Crummett, et al. and by Drs. Baughman and Meselson for measurement of dioxin through mass spectroscopy. Understandably, these results rested, apparently, on an extensive clean-up procedure. (I think some may still be bothered by what I understand is a wide intrasample variability among some of the measurements.) Since they are "pushing" their art to a point near its limits, perhaps these results deserve as much confirmation as possible. Nevertheless, the power of sensitivity and resolution are indeed impressive.

Biological Effects

I think that it is extremely important to acknowledge the fact that the original biological insight into dioxins came from a series of observations made by Dr. Suskind of accidental occupational exposures in the late 1940's. The exposure was the result of accidental release of chemical intermediates in a 2,4,5-T plant in 1949 resulting in exposure of a number of workers to manifest chloracne. In 1957, Kimmig and Schulz reported chloracne among workers in 2,4,5-T plant in Germany. A third occupational incident occurred in a 2,4,5-T plant in the United States in 1964. In a way, it is somewhat disappointing that there is not more human experience reported at this meeting. I think that there is still a clouded issue, an unclear distinction between the effects of PCB, 2,4,5-T and of dioxins and furans. For example, we should somehow ascertain whether Yusho disease in Japan was a reflection of exposure to polychlorinated biphenyls or to furan impurities. Dr. Fire-

stone reviewed the history of the contribution of the chick edema factor to our understanding of dioxins. The sleuthing done by the FDA pieced together the story of the large-scale loss of poultry (which happened first in 1957), related it to the use of tallow in poultry feed and, eventually, to the presence of dioxin impurities. Higginbotham et al. first offered a rough approximation of ranking of biological activity of dioxins which has turned out to be strikingly accurate. What has emerged from this meeting is the very wide range of toxicity for the several members of the dioxin family (perhaps as much as 10^7).

This meeting revealed some interesting (and perhaps, ultimately successful) attempts to relate chemical structure to biological activity. Several participants reminded us that to be a successful toxic dioxin, a candidate needs two halogens at the 2 and 3 positions and one at the 7 position; it also needs halogens on both benzene rings. Bromine confers more biological activity than chlorine, and chlorine more than fluorine.

I'll say very little about teratogenesis. It seems to me that a strong case can be made for clearing the air about the mechanism of teratogenesis. Is this an example of acute (embryo) toxicity with a steep dose-response curve and a demonstrable threshold? It's not clear that everyone who reports birth defects is talking about the same phenomenon. Dr. Moore, at this meeting, described some fascinating, postnatal effects of maternal exposure to TCDD through a series of cross-fostering and reciprocal cross-fostering studies of mice. These deserve further attempts at interpretation.

The effects on experimental animals are difficult to summarize completely. However, there are some underlying currents showing through:

(1) there is a variation in susceptibility among species—guinea pigs versus rats and mice; (2) there are striking sex differences; (3) delayed effects are very prominent (liver changes 2 weeks after exposure); (4) the major sites of toxic action appear to be the

liver (seen as a variety of changes in liver function), the hematopoietic system (platelet depression, altered platelet function, leucocytosis and hemoconcentration) and the lymphatic system (spleen, thymus and lymph nodes). The atrophy of the thymus and the general lymphoid depletion reported at this meeting were very striking.

As for morphological alterations, the changes in the ultrastructure under the electron microscope are of course most interesting. Perhaps the most significant point is that the morphological alterations tend to follow and confirm the functional changes which were described independently. There seems to be a delay (perhaps on the order of 3 days) between exposure and manifest structural changes. The magnitude of the change is dose-related, and the changes are reversible with time. A particularly fascinating finding was that of multinucleated hepatic cells. Do these represent a stage of attempted regeneration and repair? Alternatively, are they possible precursors of neoplastic change? This conference presented little evidence that dioxins would induce or promote neoplastic changes in tissues.

Patterns of absorption into the organism and of distribution among organs once absorbed are beginning to emerge. Not unexpectedly, water and lipid solubility seems to emerge as a major influence, although clearly not the only one. For tetrachlorodibenzo-*p*-dioxin (high doses in male rats), the amount absorbed via the intestine from an ingested dose appears to be about 70%. The majority of the absorbed dose appears in the feces at a rate of 1–2% day and in the urine at a rate of 0.5% day. A small amount can be detected in the expired air (<0.1% day). The material resident in the organism is characteristically found in the adipose tissue and the liver. It is notably absent from certain other fatty tissues such as those of the central nervous system.

By contrast, for octachlorodibenzo-*p*-dioxin, only 5% achieves absorption. Again, a large share is found in the liver (50%) and in the adipose tissues (12%). Once in

the liver, this material apparently tends to remain resident for long periods in the liver microsomes.

This meeting served to bring together a remarkable amount of work on the effect of dioxins on cellular enzymes. This was all the more remarkable, as none of this work had even been conceived of two years ago. A number of hepatic enzymes were found to be induced and a few depressed as a result of dioxin exposure. (A general caveat was voiced over what appeared to be unusually high doses of dioxin used in some of the experiments.) The degree of induction was at times striking. The experiments revealed a dose-response relationship. Again, there was an unequivocal sex difference and a characteristic latent period between exposure and induction. Effects were often long-lasting (for example, a persistent threefold increase 38 days after exposure in one experiment). Again, lipid solubility may play a large role.

The meaning of enzyme changes is as yet unclear. There are some striking differences among species. ALA synthetase, whose activity is related to the disease, porphyria, can be induced by dioxin administration in the chick embryo but apparently not in mammals. One has the impression of being very close to some insight into mechanisms yet not close enough. The combination of enzyme induction studies and changes in cellular ultrastructure could prove very helpful.

Where do we stand on our knowledge of biological activity? For furans, we know

very little. It seems to me that we still must determine whether Yusho disease was a reflection of PCB exposure or a result of exposure to dibenzofuran or other impurity. For dioxins, we now are better equipped. However, we must now reconcile a number of somewhat paradoxical observations: (1) extraordinarily high degree of biological activity, especially for certain chemical forms (tetrachlorodibenzo-*p*-dioxin was pointed out to be the most potent small molecule toxin known); (2) striking species differences in activity; (3) sex differences; (4) biological activity falls off rapidly with changes in chemical structure; (5) latent period before toxic manifestations; (6) dose-related effects; (7) long-lasting but ultimately reversible effects.

Effects on Wildlife

Understandably there is less work here than one would like. The preliminary work reported by Bowes concerning survey of wildlife is a good model and should be continued. Preliminary results seemed to suggest a very wide variety of chemical species found in the animals examined with an uncertain role for dioxins and furans.

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